The Effect of Tyrosyl Modification on the Activity of α-Lactalbumin in the Lactose Synthetase Reaction*

(Received for publication, January 20, 1971)

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SUMMARY

The effect of nitration with tetranitromethane, iodination, and treatment with tyrosinase on the ability of bovine α-lactalbumin to act as a modifier in the lactose synthetase reaction was determined. Nitration modified tyrosine and tryptophan but not histidine, iodination modified tyrosine, tryptophan, and histidine, and tyrosinase modified tryptophan but not tyrosine. The loss of activity of α-lactalbumin in the lactose synthetase reaction was correlated closely with the loss of tyrosine in all cases. Inactive dimers and polymers of α-lactalbumin were formed during nitration and iodination.

Lactose synthetase (UDP-galactose:D-glucose 1-galactosyltransferase; EC 2.4.1.22) catalyzes the biosynthesis of lactose (Equation 1).

$$\text{UDP-galactose} + \text{glucose} \rightarrow \text{lactose} + \text{UDP} \qquad (1)$$

Then enzyme requires two proteins, a galactosyltransferase and α-lactalbumin, for significant activity (2–4). The galactosyltransferase alone will catalyze the formation of lactose at high glucose concentrations (5–6). In the presence of α-lactalbumin, the apparent K_m for glucose is lowered making it an effective substrate. The galactosyltransferase will transfer galactose to GluNAc (7) and a variety of β-glycosides, including the carbohydrate side chains of glycoproteins (8).

Recent kinetic studies have shown that α-lactalbumin acts as a protein modifier of the galactosyltransferase (9) and this interaction is dependent upon substrates. Chemical modification studies of α-lactalbumin provide an assessment of the role of the amino acid residues responsible for activity in modifying the galactosyltransferase. About 40% of the activity of α-lactalbumin remained when the three histidines were carboxymethylated by iodoacetate (10). Robbins, Holmes, and Andreotti (11) nitrated α-lactalbumin with tetranitromethane and reported that both tyrosines and tryptophans are modified with loss of activity. Modifications of the carboxyl groups of α-lactalbumin with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and glycinamide inactivated α-lactalbumin (12).

The purpose of this study was to examine the role of the tyrosyl residues in α-lactalbumin by nitration, iodination, and treatment with tyrosinase. The results indicate that the tyrosyl residues are critical to the activity of α-lactalbumin as a modifier of the galactosyltransferase in the synthesis of lactose. A preliminary report has appeared (13).

EXPERIMENTAL PROCEDURE

Materials

Tyrosinase (grade II, from mushroom, 1050 units per mg), serum albumin (type I), α-chymotrypsinogen, and ovalbumin were from Sigma. Potassium iodide and D-glucose were from Baker and tetranitromethane was from Aldrich. Bio-Gels were from Bio-Rad Laboratories, Richmond, California. Bovine α-lactalbumin was prepared from skim milk (4) and was homogenous on disc gel electrophoresis. Commercial preparations of α-lactalbumin are unsatisfactory. The galactosyltransferase was prepared from bovine skim milk (5). UDP-galactose was purchased from Calbiochem. All other chemicals were reagent grade quality.

Methods

α-Lactalbumin was assayed as previously described (14). A standard curve was prepared for each set of assays which were run at 25° in a thermoregulated Beckman model DB recording spectrophotometer. A unit of activity is the amount of α-lactalbumin required to catalyze the formation of 1 mμmole of UDP per min. The concentration of α-lactalbumin was determined by measuring the absorbance at 280 nm where 1 mg per ml was equal to 2.0.

Columns used for gel filtration studies were treated with 1% solution of dichlorodimethyl silane in benzene prior to packing. Samples were made 10% in sucrose to facilitate layering on the columns. Each column was tested for uniformity of packing and determination of void volume by passage of blue dextran (1 mg per ml). Each column was calibrated with molecular

* This research was supported in part by Grant AM-10764 from the National Institutes of Health, Grant GB-7595 from the National Science Foundation, and Grant ARS 12-14-100-9204 from the Agricultural Research Service, United States Department of Agriculture, administered by the Eastern Marketing and Nutrition Research Division, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118. Journal Article 2193 of the Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma.

‡ Work submitted in part to the Graduate College of Oklahoma State University for the partial requirements of the Ph.D. degree (1).

§ Career Development Awardee, 1 K04 GM 42396 from National Institutes of Health.

weight markers (serum albumin, ovalbumin, α-chrymotypsinogen, and bovine α-lactalbumin) and there was a linear relationship between log molecular weight and elution volume. Chromatography was at 25° and flow rates were controlled with a LKB peristaltic pump utilizing a slight negative pressure. Dialyses were at 4°.

Disc gel electrophoresis was done according to instructions with the Canalco model 6 instrument. Gels were previously electrophoretically treated for 30 min at 5 ma per gel and protein separations were at 5 ma per gel. Gels were stained with aniline blue black and destained electrophoretically. α-Lactalbumin or its derivatives were extracted from stained gels by the following procedure to provide material for amino acid analysis. Separate bands, stained lightly from about 12 (native) or 50 (nitrated) discs, were cut out and soaked in 10 ml of 0.1 M NH_4OH at 37° for 3 hours with shaking. The mixture was cooled rapidly to 0° in ice and the supernatant liquid contained the dye and α-lactalbumin. The process was repeated with 10 ml of water and then 10 ml of 0.1 M NH_4OH until all the dye was extracted. The combined supernatant solutions were evaporated to dryness at 40°, dissolved in 5 ml of water, and evaporated to dryness. The samples were ready for hydrolysis for amino acid determinations. Control experiments showed the aniline blue black did not interfere with hydrolysis of α-lactalbumin for amino acid analysis. Size and charge isomers of α-lactalbumin were determined on disc gel electrophoresis by varying the per cent gel according to Hendrick and Smith (15).

Samples of α-lactalbumin for amino acid analysis were hydrolyzed with 6 N HCl in a vacuum for 24 hours at 110° and each contained 1 μmole of norleucine as an internal standard and 4% thioglycolic acid to prevent tryptophan destruction (16). It is important to add the thioglycolic acid prior to the HCl and to maintain the vacuum at less than 20 μm. Under these conditions about 90% (85 to 95) of the tryptophan is recovered in α-lactalbumin. A control of native α-lactalbumin was used for each set of experiments to correct for the destruction. Basic hydrolyses were performed in a vacuum in 0.1 ml of 5 N NaOH for 16 hours at 110°. After neutralization with 0.1 ml of 5 N HCl the sample was dissolved in 4.8 ml of the standard pH 2.2 buffer. Amino acid analyses were conducted on a Beckman model 120C amino acid analyzer.

Nitration of α-Lactalbumin—α-Lactalbumin was nitrated with a 64 molar excess of tetranitromethane (17). The reaction was stopped by lowering the pH to 4.6 with sodium acetate and the reagents were removed by chromatography on Bio-Gel P-10 or exhaustive dialysis against distilled water. The extent of nitration was determined by amino acid analysis for the appearance of 3-nitrotyrosine and the disappearance of tyrosine. No dinitrotyrosine was observed.

Iodination of α-Lactalbumin—Separate 10-mg samples of α-lactalbumin in 0.5 ml of 0.2 M Tris, pH 8.5, were iodinated at varying iodine to α-lactalbumin ratios (1:40) by the slow iodination method of Covelli and Wolff (18). After iodination the samples were kept at 4° for 30 min and then 15 μl of a 1.0 M $K_2S_2O_3$ were added to stop the reaction. The samples were dialyzed against distilled water and lyophilized.

Reaction of α-Lactalbumin with Tyrosinase—α-Lactalbumin, 12 mg, was incubated at 37° with 8 mg of tyrosinase in a final volume of 2.0 ml containing 50 mM Tris, pH 7.3. Oxygen consumption was determined on a Gilson respirometer and the reaction was stopped by chromatography on a Bio-Gel P-100 column (0.6 × 95 cm) which was equilibrated and eluted at 25° with 50 mM Tris, 0.1 M KCl, pH 7.5. The modified α-lactalbumin eluted as a single symmetrical peak which corresponded to the position of native α-lactalbumin. There was good correspondence between activity and the protein profile. The tubes containing the modified α-lactalbumin were pooled, dialyzed against distilled water, and lyophilized.

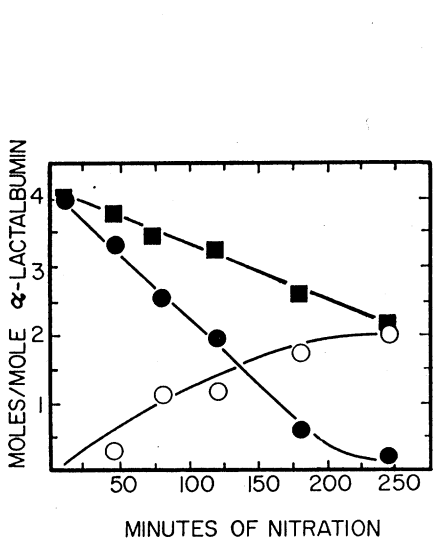

FIG. 1 (*left*). Time course of nitration of α-lactalbumin. Ten milligrams of α-lactalbumin were nitrated (see "Methods") and aliquots were removed for amino acid analysis at various time intervals. ●, disappearance of tyrosine; ○, appearance of 3-nitrotyrosine; ■, sum of tyrosine and 3-nitrotyrosine.

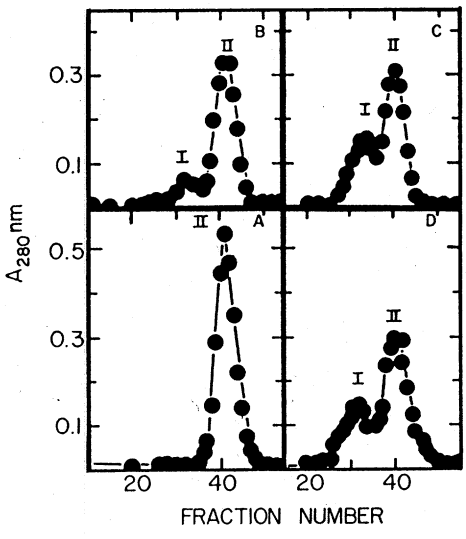

FIG. 2 (*center*). Chromatography of nitrated α-lactalbumin on Bio-Gel P-60. Columns (1.4 × 45 cm) were equilibrated and eluted with 0.1 M Tris, pH 7.4, at 25°. Each fraction contained 0.9 ml. *A*, native α-lactalbumin; *B*, α-lactalbumin nitrated for 110 min; *C*, α-lactalbumin nitrated for 180 min; and *D*, α-lactalbumin nitrated for 240 min.

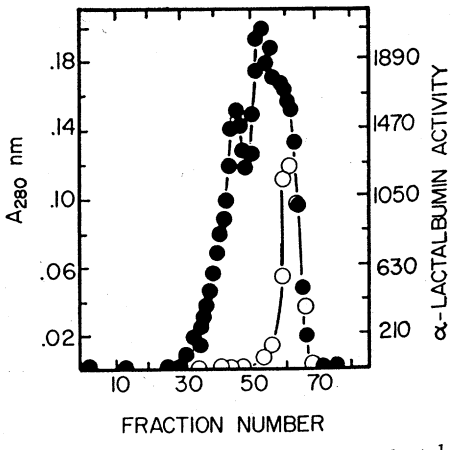

FIG. 3 (*right*). Lactose synthetase activity of nitrated α-lactalbumin eluted from a Bio-Gel P-100 column. An α-lactalbumin solution (0.3 ml of a 10 mg per ml solution) nitrated for 120 min was lyophilized and dissolved in 0.1 ml of 50 mM Tris, 0.1 M KCl, pH 7.5, and placed on a Bio-Gel P-100 column (0.6 × 95 cm). The column was eluted with 50 mM Tris, 0.1 M KCl, pH 7.5 at 25°, and 0.2-ml fractions were collected. ●, absorbance at 280 nm; ○, lactose synthetase activity in total units per fraction.

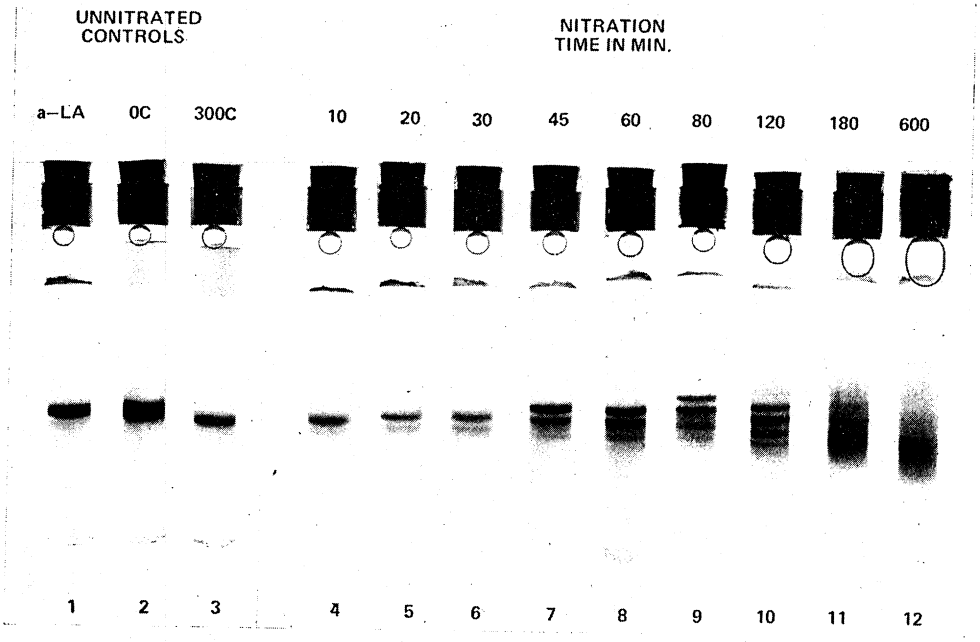

FIG. 4. Disc gel electrophoretic patterns of α-lactalbumin nitrated for various time periods. The electrophoresis was performed on standard 7% gels (see "Methods"). *Tube 1*, native α-lactalbumin (*α-LA*); *Tube 2*, zero time control (*0C*). The reaction was stopped immediately after the addition of the reagents; *Tube 3*, 300-min control (*300C*). All of the reagents except tetranitromethane were present and the reaction was stopped at 300 min. Similar results were obtained at 600 min; *Tubes 4* to *12* were nitrated for varying times as indicated above each tube after which the reaction was stopped.

RESULTS

Nitration of α-Lactalbumin—The rate of disappearance of tyrosyl residues is greater than the appearance of 3-nitrotyrosine (Fig. 1) as a function of time of nitration. At 250 min of nitration all of the 4 tyrosyl residues have disappeared whereas only 2 residues of 3-nitrotyrosine are present. Amino acid analysis showed that a new peak occurred at 58 min on the basics column and it increased as the extent of nitration increased.

Samples of α-lactalbumin nitrated for different time periods were chromatographed on Bio-Gel P-60 (Fig. 2) and a higher molecular weight material was evident which increased with the extent of nitration. Molecular weight determinations from the Bio-Gel P-60 column showed that the lower molecular weight peak (*II*, Fig. 2*D*) was 16,000 as compared to 14,500 for native α-lactalbumin and the higher molecular weight peak was (*I*, Fig. 2*D*) 37,500. In order to increase the resolution, a sample of α-lactalbumin nitrated for 120 min was chromatographed on a Bio-Gel P-100 column. The protein elution pattern and lactose synthetase activity are shown in Fig. 3 which showed that the lactose synthetase activity was present as a symmetrical peak and it coincided with the elution position of native α-lactalbumin. It would appear that nitration might be causing both a change in shape and molecular weight of α-lactalbumin. To investigate this point further, α-lactalbumin nitrated for various time intervals, was examined by gel electrophoresis and the results are shown in Fig. 4. The nitrated samples gave rise to two to five different bands (tubes *4* to *10*). Two bands are visible in tube *4*, the upper band is α-lactalbumin and the second band increases in intensity as nitration proceeds, and at tube *7* three bands are present, four in tube *8*, and five are present in tube *9*. Diffuse areas appear in tubes *10* to *12*. Attempts were made to separate the various bands in order to determine the predominant species. α-Lactalbumin nitrated for 120 min was separated into two peaks by DEAE-cellulose chromatography (Fig. 5). Each of the peak tubes, from Peaks *I* and *II*, give a single band on disc gel electrophoresis and Peak *I* was native α-lactalbumin whereas Peak *II* was nitrated α-lactalbumin as judged by amino acid analysis. The specific activity of the protein in Peak *I* was 1300 units per mg whereas that in Peak *II* was 935 units per mg (about 75% of that in Peak *I*).

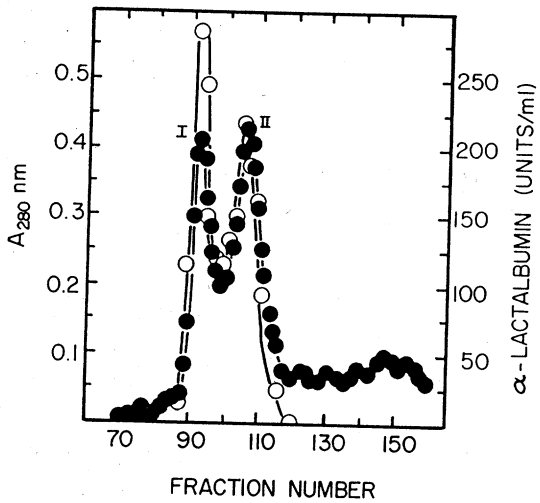

FIG. 5. Separation of nitrated α-lactalbumin on a DE-32 column. α-Lactalbumin (10 mg) was nitrated for 210 min. The reaction was stopped and the reagents were removed by dialysis against distilled water and the α-lactalbumin solution was placed on a DE-32 column (1.5 × 7 cm) equilibrated with 5 mM glycine, pH 9.5. The column was eluted at 25° with a linear gradient (250 ml each) of 5 to 700 mM glycine, pH 9.0, and 2.5-ml fractions were collected. ●, absorbance at 280 nm; ○, α-lactalbumin lactose synthetase activity in units per ml. The specific activity of Fraction 92 was 1300 and that of Fraction 106 was 935.

α-Lactalbumin nitrated for 120 min (five bands) was subjected to disc gel electrophoresis as a function of the per cent gel. The data of R_m *versus* per cent gel gave a series of parallel lines (15) indicating that the proteins were charge isomers (Fig. 6). The protein from the various stained bands from 50 separate disc gels of α-lactalbumin nitrated for 120 min were isolated from the disc gels (see "Methods") and subjected to amino acid analysis. The first or slowest moving band was native α-lactalbumin and contained 4.3 moles of tyrosine per mole, the second contained 3.1, and the third contained 2.2 moles of

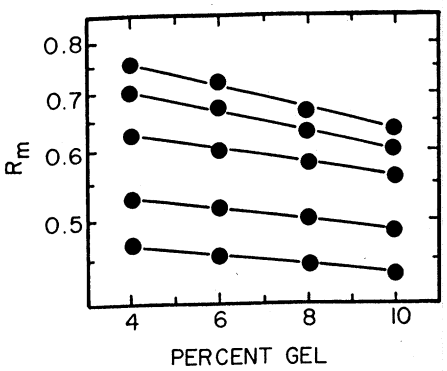

FIG. 6. Ratio of the logarithm of protein migration to dye migration (R_m) of α-lactalbumin nitrated for 120 min on gels varying in the percentage of acrylamide.

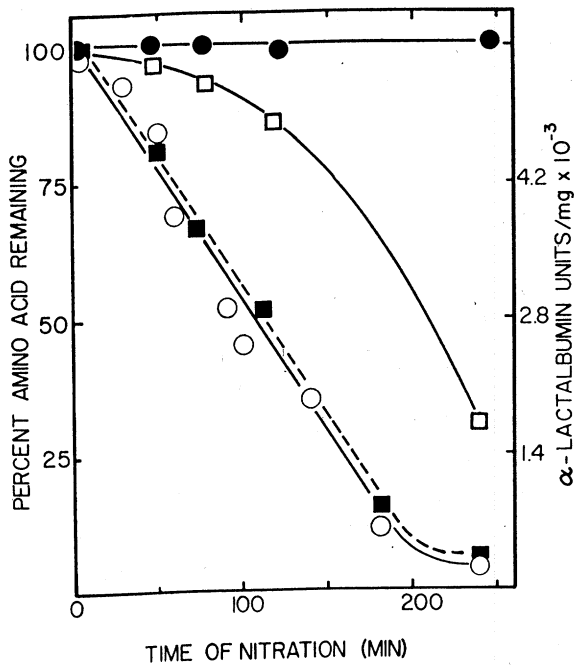

FIG. 7. Effect of time of nitration on histidine, ●; tryptophan, □; and tyrosine, ■, loss from α-lactalbumin as related to activity, ○. One hundred per cent is equal to three histidines, four tyrosines, and four tryptophans.

tyrosine per mole of α-lactalbumin. Not enough material was available for analysis for the fourth and fifth bands. These results suggest that the bands represent native, mono-, di-, tri-, and tetra-, nitro-α-lactalbumin, respectively.

The specificity of the nitration reaction was monitored by complete amino acid analysis of the product in the presence of thioglycolic acid (see "Methods"). 3-Nitrotyrosine was formed but 3,5-dinitrotyrosine was absent. Tryptophan as well as tyrosine was lost during the nitration.

The effect of nitration for 240 min on the loss of histidinyl, tyrosyl, and tryptophanyl residues of α-lactalbumin as related to activity in the lactose synthetase assay is shown in Fig. 7. There was no loss of histidine but both tyrosine and tryptophan disappeared and the loss of activity closely paralleled the loss of tyrosine. After 20 min, an average of 2.7 tryptophanyl residues and all the tyrosines were lost. A plot of the log of tyrosyl loss as a function of time gave a pseudo first order rate constant equal to $k_1 = 0.33$ min^{-1} (for the first 75 to 100 min where one tyrosyl is lost) and then the rate increased $k_2 = 1.74$ min^{-1}. The initial rate of formation of 3-nitrotyrosine was $k_3 = 0.30$ min^{-1}. It appears that the first tyrosyl lost is modified to 3-nitrotyrosine but after this time a secondary and faster reaction predominates which results in formation of polymers. The results of the nitration experiments strongly implicate the essential role of the tyrosyl residues in α-lactalbumin as related to lactose synthetase activity.

Iodination of α-Lactalbumin—The nitration experiments showed that both tryptophan and tyrosine were lost and that the activity loss closely paralleled the tyrosyl loss.

The effect of iodination on loss of tryptophan and loss of activity of α-lactalbumin in the lactose synthetase reaction is shown in Fig. 8. The loss in lactose synthetase activity is more rapid than the loss of tryptophan. The relationship between the loss in activity and loss of tyrosine as determined by acid and basic hydrolysis is shown also in Fig. 8. With acid hydrolysis it would appear that only 2 tyrosyl residues were lost but control experiments with both monoiodotyrosine and diiodotyrosine showed that they were destroyed about 50% and recovered as tyrosine. Control experiments utilizing alkaline hydrolysis showed that less than 1% of the mono- and diiodotyrosine was converted to tyrosine and hence tyrosine loss is accurately monitored by alkaline hydrolysis. There was a good correlation between tyrosyl loss and loss in activity which supports the results obtained from the nitration experiments.

The first appearance of diiodotyrosine was observed at an I_2 to α-lactalbumin ratio of 12:1 and 2.5 residues of diiodotyrosine per mole were formed when the ratio was 40:1. In this procedure (19) monoiodotyrosine elutes with an interfering substance and it was shown in the present study that this was tryptophan. It is possible to determine the concentration of monoiodotyrosine by making use of the 4% thioglycolic acid. Alkaline hydrolysis gives the sum of monoiodotyrosine and tryptophan and subsequent acid hydrolysis in the presence of thioglycolic acid destroys the monoiodotyrosine but not tryptophan which is recovered from 87 to 92%.

The only other amino acid modified by iodination was histidine (Fig. 9) and the loss of histidine did not correlate with the loss of lactose synthetase activity.

Previous studies on the nitration of α-lactalbumin showed that polymer formation occurred. α-Lactalbumin iodinated at various ratios of I_2 to α-lactalbumin was chromatographed on Bio-Gel P-100. The elution patterns are presented in Fig. 10. At an I_2 to α-lactalbumin ratio of 12:1, 1.2 tyrosyls were modified but no polymer was present (Fig. 10*A*). When the ratio of I_2 to α-lactalbumin was 20:1, 2.2 tyrosyl residues were modified and polymer occurred (Fig. 10*B*) which corresponds to an α-lactalbumin dimer. The ratio of monomer to polymer is about 6.5:1. α-Lactalbumin iodinated at a 40:1 ratio of I_2 to α-lactalbumin resulted in the modification of 3.6 tyrosines, 1.1 tryptophans, and 1 histidine, and the elution pattern (Fig. 10*C*) shows the formation of a major peak which corresponds to a trimer. The activity of α-lactalbumin in the lactose synthetase reaction gave a symmetrical peak and corresponded to the position and shape of native α-lactalbumin. α-Lactalbumin, which has undergone a shape change or formed polymers, is devoid of activity which is similar to the results obtained with the nitration experiments.

The disc gel electrophoretic patterns of α-lactalbumin iodinated at a ratio of I_2 to α-lactalbumin of 40:1 gave three major bands.

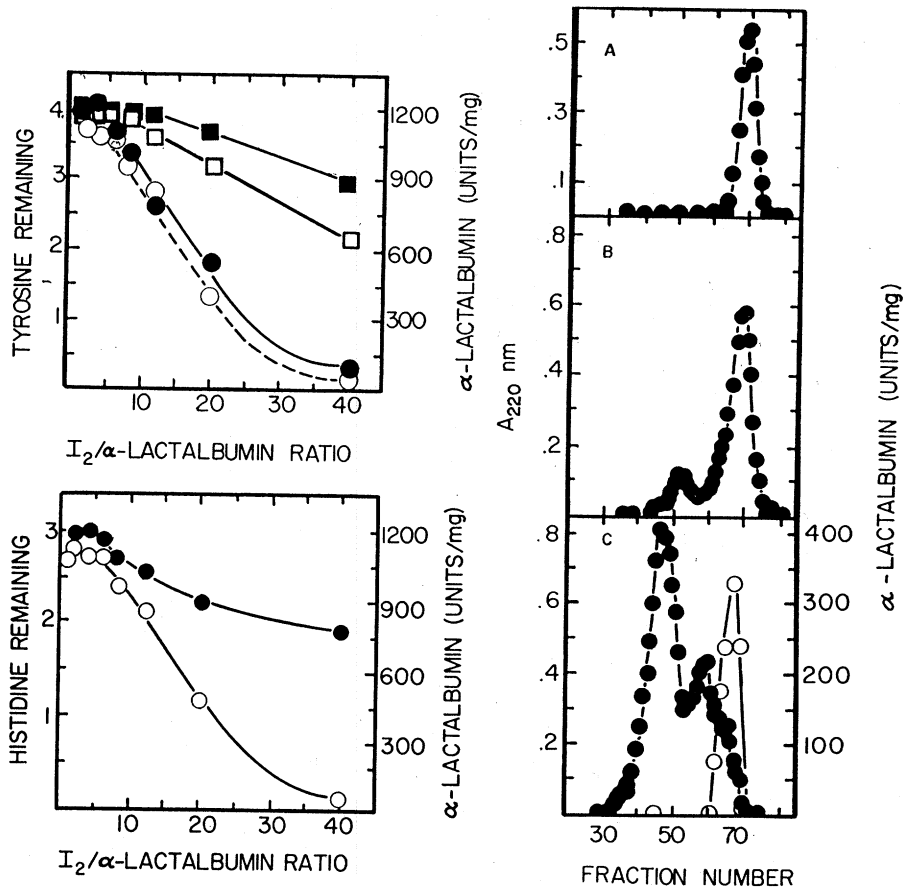

FIG. 8 (*upper left*). Effect of iodination of α-lactalbumin on tyrosyl and tryptophanyl loss and activity of α-lactalbumin ●, tyrosine remaining after alkaline hydrolysis (see "Methods"); □, tyrosine remaining after acid hydrolysis; ■, tryptophan remaining after acid hydrolysis with thioglycolic acid (see "Methods"); and ○, activity of α-lactalbumin in the lactose synthetase reaction (units per mg).

FIG. 9 (*lower left*). Effect of iodination of α-lactalbumin upon loss of histidine and activity. α-Lactalbumin was iodinated (see "Methods") and histidine loss was determined by amino acid analysis after acid hydrolysis (see "Methods"). ●, histidine residues remaining; ○, activity of α-lactalbumin in the lactose synthetase reaction (units per mg).

FIG. (10 (*right*). Bio-Gel P-100 chromatography of α-lactalbumin iodinated at (*A*) I_2 to α-lactalbumin ratio of 12:1, (*B*) I_2 to α-lactalbumin ratio of 20:1, and (*C*) and I_2 to α-lactalbumin ratio of 40:1. A Bio-Gel P-100 column (0.6 × 95 cm) was equilibrated at 25° and eluted with 50 mM Tris, 0.1 M KCl, pH 7.5, and 0.2-ml fractions were collected. Each tube was diluted to 0.8 ml with water and the absorbance was read at 220 nm.

The logarithm of the protein mobility as a function of the percentage of acrylamide gel concentration is shown in Fig. 11. A complex pattern was observed and the parallel lines (Fig. 11) are indicative of charge isomers whereas the converging lines represent weight isomers. The three major bands in the disc gels appear to represent native α-lactalbumin and a charge and weight isomer.

The loss of lactose synthetase activity is more than 5 times faster than the loss of tryptophanyl residues and more than twice the loss of histidine, but closely parallels the loss of tyrosyl residues, again implicating the functional importance of the tyrosyl residues in α-lactalbumin.

Modification of α-Lactalbumin by Tyrosinase—Oxygen uptake from 12 mg of α-lactalbumin showed that about 28 μl of oxygen were utilized during 300-min incubation with tyrosinase at 37°, which corresponds to the oxidation of about 1.5 tyrosyl residues. However, amino acid analyses of α-lactalbumin treated 300 min with tyrosinase (Table I) showed essentially no loss of tyrosyl residues but a loss of about one tryptophan. Control experiments as measured by oxygen uptake showed that tyrosinase

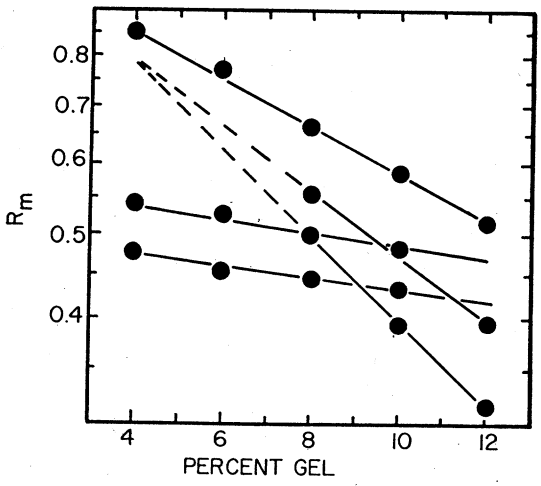

FIG. 11. Ratio of the logarithm of protein migration to dye migration (R_m) of α-lactalbumin iodinated at a ratio of I_2 to α-lactalbumin of 40:1 (see "Methods").

TABLE I

Effect of tyrosinase on amino acid composition of α-lactalbumin

An unknown occurred at 58 min in the elution pattern and probably is a tryptophan degradation product (0.01 μmole was formed compared to 0.039 μmole of phenylalanine). Experiments 2a and 2b are duplicate analyses.

Amino acid	Native α-lactalbumin		Tyrosinase-treated α-lactalbumin, percentage of recovery		
	No. of residues	Recovery	Experiment 1	Experiment 2a	Experiment 2b
		%	%	%	%
Histidine	3	101	91	107	107
Tryptophan	4	100	64	72	72
Tyrosine	4	101	95	97	94
Phenylalanine	4	100	100	100	100

rapidly oxidized tyrosine but slowly oxidized tryptophan and histidine at rates comparable to α-lactalbumin. Boiling tyrosinase in 50 mM Tris, pH 7.3, for 10 min did not always denature the enzyme completely.

Gel filtration of α-lactalbumin treated 300 min with tyrosinase on Bio-Gel P-100 gave a single major peak at the position of native α-lactalbumin and activity in the lactose synthetase system corresponded to the protein peak. At various time intervals, samples of α-lactalbumin incubated at 37° in the presence of tyrosinase were removed and separated from tyrosinase by filtration on a Bio-Gel P-30 column. α-Lactalbumin treated 300 min with tyrosinase retained 95% of its activity in the lactose synthetase reaction.

The results show (Table I) that tyrosinase does not oxidize the tyrosyl residues of α-lactalbumin but instead oxidizes 1.1 to 1.5 tryptophan residues under these conditions and still retains 95% of its activity in the lactose synthetase reaction.

DISCUSSION

Inspection of the model of α-lactalbumin based on the coordinates of lysozyme (20) shows that all four tyrosyls of α-lactalbumin are located on or near the surface of the molecule. Robbins *et al.* (11) showed that the four tyrosyls ionize normally, and Gorbunoff (21) reported that they react readily with *N*-acetylimidazole and at least three of the four tyrosyls react readily with cyanuric fluoride. The present study on nitration and iodination shows that all four tyrosyls are modified in a random fashion since they are equally accessible to the reagents.

During nitration, four tyrosyls are lost but only 2 moles of nitrotyrosine are formed per mole of α-lactalbumin. Atassi, Habeeb, and Rydstedt (22) reported that 2.51 ± 0.03 tyrosines of α-lactalbumin were nitrated with tetranitromethane, but Robbins *et al.* (11) showed that all four tyrosyls were modified although 2 residues were apparently more reactive than the others. Polymer formation was observed in both the nitration and iodination experiments and these polymers were inactive in the lactose synthetase reaction. The extent of dimer and polymer formation varied with the experiment and there was no direct correlation between polymer formation and loss of activity although this is difficult to ascertain since apparent shape changes occur after 1 residue is modified by nitration or iodination. The polymer formation may represent cross-linked products formed during the reaction and similar results have been observed with insulin and glycyl-L-tyrosine (23).

Sokolovsky and Riordan (24) have shown that stopping the nitration reaction at pH 4.0 causes the formation of nitrite and with ribonuclease additional tyrosine is destroyed so that the sum of tyrosine plus nitrotyrosine is less than expected. Most of the nitration reactions in this study were stopped at pH 4.6. Additional experiments showed that when the reaction was stopped at pH 7.5 by dialysis or chromatography on Bio-Gel P-10 dimer and polymer formation still occurred.

The present study is not in agreement with earlier work where it was reported that tyrosinase oxidized the tyrosyl residues of α-lactalbumin (25). Tyrosinase does not oxidize the tyrosyls of lysozyme (26). Oxygen uptake experiments with tryptophan and histidine as substrates have shown that the rate of oxygen uptake with these amino acids is approximately similar to the rates observed with α-lactalbumin, and it would appear that the tyrosyls of α-lactalbumin are not functioning as a substrate for tyrosinase.

The nitration experiments showed that tyrosine and tryptophan were modified but not histidine; the results of the iodination experiments showed tryptophan, tyrosine, and histidine were modified and the results of the tyrosinase experiments showed that the tryptophan was modified with essentially no loss of activity. In all cases, the loss of activity of α-lactalbumin in the lactose synthetase reaction closely paralleled the loss of tyrosyls whereas the loss of histidine and tryptophan are not directly correlated with the loss of activity (27). Thus, the tyrosyl residues of α-lactalbumin appear to be essential for activity in the lactose synthetase reaction.

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